

Artigo Original

Lack of association between ACE ID genetic polymorphism and diabetes or hypertension in Brazilians aged from 50 to 70 years old

Falta de associação entre o polimorfismo ID do gene da ECA e diabetes ou hipertensão em brasileiros com idade entre 50 a 70 anos

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ABSTRACT

The present study examined the association between ACE ID polymorphism and diabetes or hypertension in individuals aged between 50 to 70 years old. The participants were recruited before initiating a physical training program and divided into groups, according to their genotype of the ACE gene; DD (n=40), ID (n=55), or II (n=31). The anthropometrical characteristics of the participants were evaluated and anamneses performed to establish if the participants presented diabetes and/or hypertension. From the data obtained for anthropometric characteristics, diabetes, and hypertension, we did not find any association with the ACE ID genotypes (DD, ID and II). ACE ID polymorphism may not have an association with diabetes or hypertension in the 50 to 70 years old Brazilian population, but it might have an association in other populations with different ages.

Keywords: Chronic Disease. Aging. Health. Genetic Polymorphisms.

RESUMO

O presente trabalho verificou a associação entre o polimorfismo ID do Gene. da ECA e diabetes ou hipertensão em indivíduos de 50 a 70 anos. Os Participantes foram recrutados antes de iniciarem em um programa de atividade física e foram divididos em grupos, de acordo com genótipo para o Gene. da ECA; DD (n=40), ID (n=55), ou II (n=31). Dados antropométricos dos participantes foram coletados, e também responderam uma anamnese afim de estabelecer se possuíam ou não diabetes e/ou hipertensão. Para os dados antropométricos, diabetes e hipertensão, não foi encontrado associação com os genótipos (DD, ID e II) para o Gene. da ECA. O polimorfismo ID do Gene. da ECA pode não ter associação com diabetes ou hipertensão na população de 50 a 70 anos brasileira, mas pode ter associação em indivíduos de outros países e de diferentes idades.

Palavras chave: Doença Crônica. Envelhecimento. Saúde. Polimorfismo Genético.

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Introduction

Aging is a dynamic, progressive, and irreversible process that forms part of human life. This process is related to biological, social, and psychological declines.¹ Aging is related to an increase in the prevalence of chronic diseases, such as hypertension and diabetes. These diseases are associated with a worse life quality in older people, and environmental and genetic factors can affect both diseases.²

Type 1 diabetes mellitus is a result of immune disorders, and is characterized by destruction of pancreatic islet β cells and insulinitis, resulting in a deficient in or lack of insulin secretion.³ Type 2 diabetes mellitus is characterized by chronic high levels of blood glucose and altered levels of insulin, resulting in insulin resistance or insulin insufficiency in skeletal muscle, the liver, and adipose tissue.⁴ Hypertension is characterized by systolic blood pressure higher than 140 mmHg and/or diastolic blood pressure higher than 90 mmHg. The prognosis of these diseases is still unknown, but older age and obesity are the two main risk factors.⁵

The ACE enzyme acts by converting angiotensin I into angiotensin II, which has a vasoconstrictor role. The genetic polymorphism (rs 1799752) in the ACE gene is responsible for an insertion (I allele) or deletion (D allele) of 287pb,⁶ which the D allele results in a higher Gene. expression, and seems to be associated with increased risk for diabetes and hypertension.^{7,8} In addition, higher level of ACE in normotensive diabetes-induced mice resulted in increased blood pressure and nephropathy.⁹ To our knowledge, there are few studies, mentioned below in the discussion, regarding the association of the frequency of ACE ID genotype with hypertension and diabetes in older subjects, still further in Brazilian older individuals.

Evaluating the association of ACE ID genotype frequency and chronic diseases in older individuals is important to predict which is associated with these diseases in order to target the relevant portion of the population before they reach old age to improve their life quality and possibly avoid the diseases. In this regard, we aimed to evaluate the association between the ACE ID genetic polymorphism and hypertension or diabetes.

Methods

Participants

Older participants aged between 50 to 70 years old, before initiating in an exercise training program were invited to participate in this research. The exclusion criteria of the exercise training program were present cardiovascular disease or risk factors for cardiovascular disease or other diseases without to present medical certificate to physical exercise. The participants signed a written informed consent. The experimental procedure was approved by the Ethics Committee of the Faculty of Philosophy Sciences and Languages of Ribeirão Preto – FFCLRP (University of São Paulo USP CAAE: 24579513.4.0000.5407).

Genotype assessment

A peripheral blood sample was collected in EDTA tubes and the DNA was extracted using the *salting out* method.¹⁰ The ACE I/D polymorphisms (rs 1799752) were amplified by the polymerase chain reaction (PCR) and the products from the amplification were genotyped by electrophoresis in agarose gel. The primers used were F-5'-CTGGAGACCACTCCCATCCTTTCT-3' and R-5'-GATCTGGCCATCACATTCGTCAGAT-3'. The PCR conditions were: initial denaturation at 95°C for 3 min, 35 cycles at 95°C for 30 s, 58°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 10 min. The fragments with the insertion (allele I) of 287 pb result in an amplicon of 478 bp, and the fragments without the insertion (allele D) result in an amplicon of 191pb. Fragments were detected on 1.5% agarose gel containing ethidium bromide.^{11,12}

Measurements

Participants were evaluated for the following parameters: body mass (precision 50g), and height using a stadiometer (precision 1.0 mm) (*Welmy W200LCD*), and body mass index - BMI (weight divided by height squared).¹³ In addition, an anamnesis was performed to establish if the participants presented diabetes or hypertension. We also applied the International Physical Activity Questionnaire (IPAQ).¹⁴ All data were collected in the initial evaluation of the training program.

Statistical analyses

Individuals were divided into groups according to their genotype. The data were analyzed by one-way ANOVA. *Bonferroni's* post hoc test was used for ANOVA differences. For the qualitative analyses, the chi-square test was used. The Hardy-Weinberg Equilibrium was calculated using the χ^2 test. The adopted significance level was $p \leq 0.05$. The data were analyzed using *SPSS* 21.0.

Results

The genotype distribution for the ACE gene was DD $n = 40$ (31.75%), ID $n = 55$ (43.65%), and II $n = 31$ (24.60%). No significant differences was observed in the Hardy-Weinberg Equilibrium ($p=0.169$).

The data for age, height, weight, and BMI are presented in table 1. There were no differences between the groups for these variables. Table 2 presents data on the relationship between diabetes, hypertension, and the ACE genotypes. No association between the ACE genotypes and diabetes or hypertension was found.

Discussion

In the present study, we aimed to investigate the association between the ACE ID genotypes and diabetes or hypertension.

Table 1 presents the anthropometrical characteristics. No association was found with the ACE ID genotypes. Pereira et al.,¹⁵ in a study with 139 older Caucasian women, 65.5 (8.2) years, did not find differences between the genotypes for weight

Table 1: Sample characterization

| | II N= 40 | ID N= 55 | DD N= 31 |
|-------------------------------|---------------|---------------|---------------|
| Age (years) | 61.26 (6.25) | 60.60 (5.46) | 60.00 (5.25) |
| Height (m) | 1.60 (0.08) | 1.61 (0.07) | 1.60 (0.05) |
| Weight (kg) | 77.30 (17.69) | 75.51 (18.32) | 71.56 (11.50) |
| BMI (kg/m²) | 30.45 (6.34) | 28.98 (6.40) | 27.88 (4.17) |
| Walking (min/week) | 180.4 (194.8) | 193.5 (176.1) | 186.2 (187.0) |
| Moderate PA (min/week) | 236.4 (337.0) | 379.0 (527.4) | 382.9 (455.6) |
| Intense PA (min/week) | 61.0 (198.5) | 97.9 (244.2) | 32.9 (73.6) |

Data are presented as mean and standard deviation in parentheses; BMI: body mass index.
PA: Physical activity

Table 2: Association between diabetes, hypertension and ACE genotypes

| | Diabetes | | | Hypertension | | |
|----------------|----------|-------|-------|--------------|-------|-------|
| | Yes | No | P | Yes | No | P |
| DD N=40 | 85% | 15% | 0.350 | 52.5% | 47.5% | 0.341 |
| ID N=54 | 79.6% | 20.4% | | 48.1% | 51.9% | |
| II N=31 | 71.0% | 29.0% | | 64.5% | 35.5% | |

Data are presented as number of participants in percentage that have or do not diabetes or hypertension according to their genotype.

or BMI. Keogh et al.,¹⁶ in a study with Australian older woman, 69.8 (4.9) years, did not observe any differences for weight or BMI between the groups. Lemes et al.,¹⁷ in a study with Brazilian children and adolescents between the ages of 7 and 16 years did not find any association between weight and the genotypes, however the authors observed an association of D allele with higher BMI values for boys. Kim¹⁸ demonstrated that Korean adult women aged 38.0 (1.0) years, presented no association between the ACE genotypes and anthropometric characteristics. Thus, the ACE ID genotype seems not to be associated with anthropometric characteristics in older adults or adults, although it may have an association in children. In addition, our data show that the groups in the present study were similar, suggesting that anthropometric characteristics did not affect the condition of the groups for diabetes or hypertension.

With regard to diabetes and hypertension, we did not find any association between the ACE genotypes and hypertension or diabetes, as demonstrated in Table 2. The ACE enzyme is responsible for converting angiotensin I into angiotensin II, which is a potent vasoconstrictor. In addition, it seems to be related to diabetes as angiotensin II is an inflammatory adipocytokine and an increase in the enzyme activity results in glucose storage.¹⁹

Shaikh et al.,²⁰ in a study with 110 healthy participants and 115 with diabetes mellitus demonstrated a higher D allele frequency in the group with diabetes when compared to the control. Baroudi et al.,²¹ studying the association between the ACE ID genotype and type 2 diabetes in older Jerbian individuals, demonstrated a higher DD genotype frequency in the diabetes group than the control group. On the other hand, Wollinger et al.,²² researching the older Brazilian population, demonstrated a lack of association between the ACE genotypes and type 2 diabetes.

Related to hypertension, Shanmuganathan et al.,²³ demonstrated an association between the ACE genotypes and hypertension in the Indian Population. In the above previously mentioned study, Kim¹⁶ found only a trend for the association between hypertension and ACE genotypes. In contrast, Arfa et al.,²⁴ in a study with Tunisian subjects, did not found any association between hypertension and ACE genotypes.

As a limitation of the present study, we can mention the evaluation of the presence or not of diabetes and/or hypertension only through anamneses, which may result in diagnosis mistakes. There is a possibility that some participants did not know about their clinical diagnosis. However, all participants were evaluated by a doctor prior to the study, which should have helped to avoid errors due to misdiagnosis.

Furthermore, to utilize other methods in the evaluation would have resulted in fewer participants in the study due to the use of invasive methods. In addition, as a further limitation, the expression level of ACE was not evaluated which could have increased the impact of our results.

Conclusion

In conclusion, this research demonstrated that there might not be any association between the ACE genotypes and diabetes and hypertension in the older Brazilian population. However, our results are different from others in the literature with different ethnical groups. Thus, further studies are necessary to reach a conclusion about the relationship between ACE ID genotypes and diabetes and hypertension.

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